Array Comparative Genomic Hybridization of Keratoacanthomas and Squamous Cell Carcinomas: Different Patterns of Genetic Aberrations Suggest Two Distinct Entities

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Keratoacanthoma (KA) is a benign keratinocytic neoplasm that spontaneously regresses after 3–6 months and shares features with squamous cell carcinomas (SCCs). Furthermore, there are reports of KAs that have metastasized, invoking the question of whether KA is a variant of SCC (Hodak *et al.*, 1993). To date, no reported criteria are sensitive enough to discriminate reliably between KA and SCC, and consequently there is a clinical need for discriminating markers. Our previous study analyzed 132 KAs and 29 SCCs and revealed significantly different regions of genomic aberrations using chromosomal comparative genomic hybridization (CGH). In the present study, we applied array CGH to investigate 98 KAs and 22 SCCs from the above samples. The result shows that all KAs and SCCs have some degree of genetic aberrations. The distribution of numbers of aberrant clones per sample differed significantly between KAs and SCCs ($P<0.02$), which also demonstrated recurrent aberrations that differed significantly ($P<0.001$), as illustrated by unsupervised cluster analysis. Classifiers for clinicopathological parameters of KAs were established based on *t*-test statistics and permutation tests. Tumor size, fibrosis, and inflammation, which are related to the developmental stages of KAs, showed significant (*t*-test, permutation test) associations with aberrations of selected genomic regions. This suggests chromosomal instability during the whole life cycle of KAs.

Journal of Investigative Dermatology (2012) **132,** 2060–2066; doi:10.1038/jid.2012.104; published online 26 April 2012

INTRODUCTION

Keratoacanthoma (KA) is a common, unique benign keratinocytic neoplasm that usually presents as a solitary nodule developing a central keratin-filled crater. The lesion is usually located on sun-exposed areas of the skin of elderly persons. It mostly develops within 6–8 weeks, with spontaneous regression after 3–6 months, although much longer life spans are also observed. Thus, KAs typically evolve in three clinical stages: the proliferative, maturing, and resolving stage. KAs may appear as multiple lesions (Grzybowski, 1950; Muir *et al.*, 1967; Ferguson-Smith *et al.*, 1971), and the incidence is increased among immunosuppressed patients (Sullivan and Colditz, 1979). The histopathological diagnosis of KA is based on the architecture and on cytologic features, and when a typical clinical history is known most KAs can be distinguished from squamous cell carcinomas (SCCs) (Elder *et al.*, 1997; Weedon, 2003). However, particularly in the early proliferative phase, KA shares features with SCC such as infiltration and cytological atypia and is considered by some to be an abortive or self-healing malignancy (Schwartz, 1994). However, the literature also reports KAs that have metastasized, raising the question of whether KA is a variant of SCC (Hodak *et al.*, 1993). The discussions on this issue of KAs are still ongoing (Schwartz, 2004; Karaa and Khachemoune, 2007; Mandrell and Santa Cruz, 2009; Ko, 2010; Weedon *et al.*, 2010a).

So far, there are no criteria that are sensitive enough to discriminate reliably between KA and SCC (Kerschmann *et al.*, 1994; Quinn *et al.*, 1994; Cribier *et al.*, 1999; Jensen *et al.*, 1999; Putti *et al.*, 2004). There is thus a clinical need for markers that may distinguish between the two entities. Previously, Clausen *et al.* (2002, 2006) found, by means of comparative genomic hybridization (CGH), that about one-third of KAs harbored genomic aberrations, and that

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Abbreviations: BAC, bacterial artificial chromosome; CGT, comparative genomic hybridization; KA, keratoacanthoma; SCC, squamous cell carcinoma

Received 6 August 2011; revised 15 February 2012; accepted 16 February 2012; published online 26 April 2012

cutaneous SCCs had a significantly higher degree of chromosomal aberrations, and thus chromosomal instability, than KAs.

Array-based CGH (array CGH) is a global DNA analysis method where labeled DNA fragments are hybridized to arrays of bacterial artificial chromosome (BAC) clones instead of metaphase chromosomes, as used in the conventional CGH method (Snijders *et al.*, 2001). The 1-Mb-resolution BAC clone set used in the present study comprises 3,340 clones, and is a much more sensitive method for screening genomic aberrations than conventional CGH.

The aim of the present study was 2-fold. First, we wanted to characterize genomic aberrations with the more sensitive technique array CGH in different developmental stages and categories of KAs. Second, we wanted to compare genetic aberrations found in KAs and SCCs to analyze whether the two types of neoplasia are interrelated or whether they constitute two separate entities.

RESULTS

Clinicopathological description of KAs is shown in Supplementary Table S1 online. All analyzed samples of KAs, as well as of SCCs, harbored genetic aberrations when analyzed by array CGH (Supplementary Table S2 online). An overview of the genetic aberration profiles for all chromosomes except for Y is shown for KAs in Figure 1a, and for SCCs in Figure 1b. Regions with gains (dark gray and above the zero line), as well as with losses (light gray and below the zero line), are to a large extent overlapping for KAs and SCCs. However, there are also obvious differences in the patterns of aberrations between the two types of neoplasms—as seen most clearly on chromosomes 7, 8, and 10. The size of the regions with detected aberrations was summed up for each tumor, and the means for KAs and SCCs were found to constitute 17.2% and 24.9% of the whole genome, respectively. In Figure 2, KAs and SCCs show two main clusters in a dendrogram using unsupervised cluster analysis, also demonstrating that the aberration profiles of these two types of lesions are significantly different (Fisher's exact test, $P<0.001$). The same tendency of differential distribution of KAs and SCCs in the dendrogram is also seen in pvclust analysis (Fisher's exact test, $P=0.02903$) (Supplementary Figure S1 online). Furthermore, the aberrant clone numbers per sample in the two types of neoplasms were significantly different (Wilcox–Man–Whitney test, $P<0.02$), with the majority of KAs having fewer aberrant clones as illustrated in Figure 3. However, some large recurrent aberrations in KAs were found (Supplementary Table S3 online). Most of chromosome 19 (19p13.3–19q13.43) was lost in 37% of the cases, and gains of most of chromosome X (Xp22.33–Xq28) were found in a similar number of cases. Large aberrations (gain) were also found in chromosome 20 (20p13–20q13.33) in 34% of the cases. In the SCCs, gains in 8q11.22–8q24.3, 7p22.2–7q36.3, and Xp22.33-Xq28 were detected in 35%, 34%, and 35% of the cases, respectively, whereas losses in 10p15.3–10q26.3 and13q12.11–13q34 were detected in 34% and 38% of the cases, respectively (Supplementary Table S3 online).

Chromosomal region 17p13.3–17q25.3 was lost in 27% of the cases of KAs. The same region was lost in 46% of the SCCs. The important tumor suppressor gene, *TP53*, is located in these regions, and we have recently shown (Durinck *et al.*, 2011) that loss of functional TP53 is one of the earliest and most crucial steps in the development of malignant cutaneous SCCs. Thus, we analyzed the TP53 region in more detail. In the BAC clone set, we identified two overlapping BAC clones (RP11-199F11, chr17, 7438479–7599079; RP11-404G1, chr17, 7563870–7733801) as reflecting the copy number variations for TP53, as TP53 is located in these two BAC clones (17p13.1). According to the copy number status

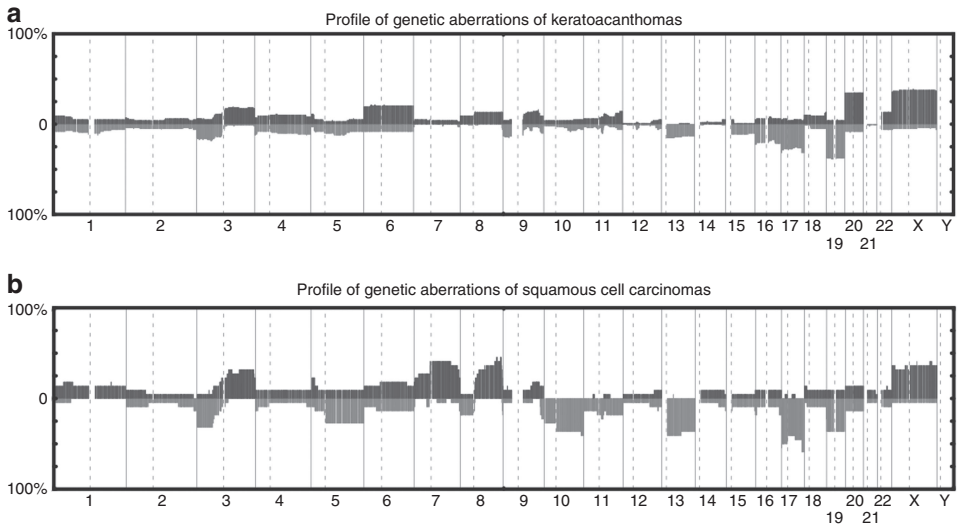

Figure 1. Profiles of genetic aberrations. (**a**) Keratoacanthomas and (**b**) squamous cell carcinomas along all chromosomes with the exception of chromosome Y; dark gray (above the zero line) indicates amplification and light gray (below the zero line) indicates loss. Note differences between the two types of lesions within chromosomes 7, 8, and 10.

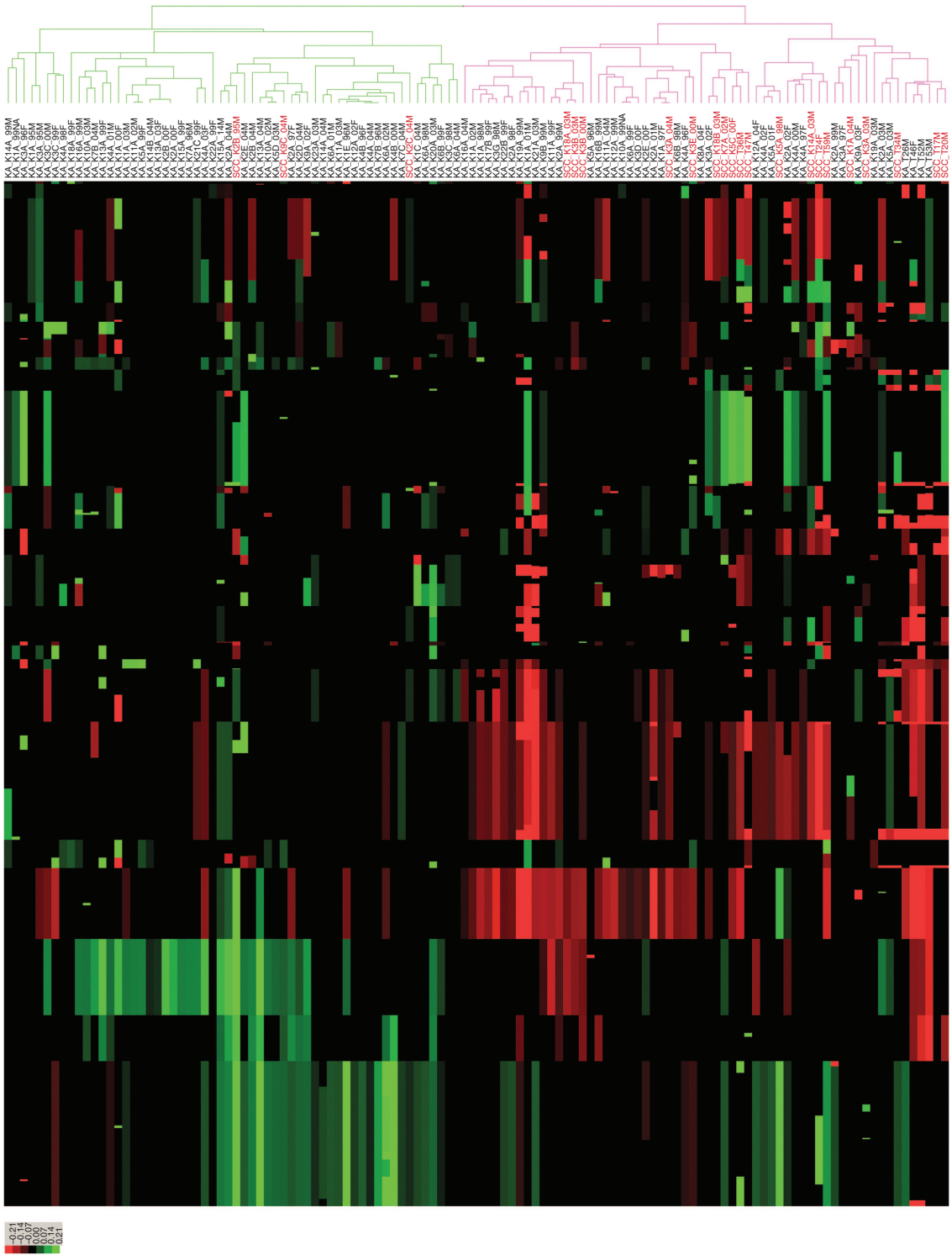

Figure 2. Unsupervised cluster analysis of squamous cell carcinomas (SCCs) and keratoacanthomas (KAs). Losses are indicated by red and gains by green. The aberration profiles of KAs and SCCs are significantly different (Fisher's exact test, $P<0.001$).

of these two BAC clones, we classified KAs and SCCs into four groups: KA with TP53 deletion ($n=28$), KA without TP53 deletion ($n=64$), SCC with TP53 deletion ($n=11$), and SCC without TP53 deletion ($n=11$). The mean of the total aberration percentage values for each group is as follows: KA with TP53 deletion (18.77%), KA without TP53 deletion (15.59%), SCC with TP53 deletion (32.49%), and SCC without TP53 deletion (17.29%). The values of aberration percentage are not normally distributed for the KA groups (overrepresentation of cases with very few or relatively many

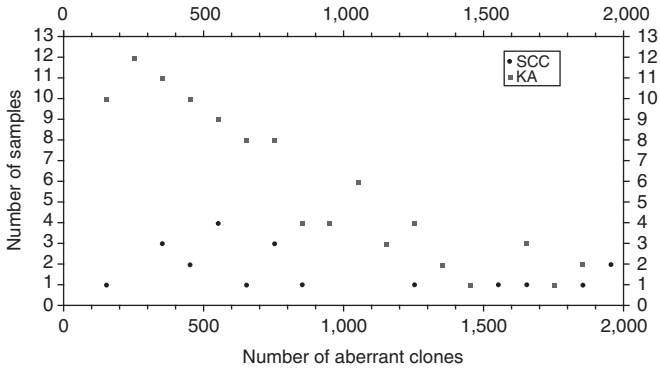

Figure 3. Distribution of numbers of aberrant clones per sample for keratoacanthomas (squares) and squamous cell carcinomas (dots). Note the higher number of samples with few aberrations among keratoacanthomas.

aberrations) but logarithmic transformation of all data allowed Welch *t*-tests for significant differences in mean aberration values between groups. KAs with and without TP53 deletion are significantly different in this respect ($P<0.0234$) and so are SCCs with and without TP53 deletion ($P<0.0192$). Furthermore, the mean aberration value in the group of SCC with TP53 deletion was larger than in the group of KAs with TP53 deletion ($P<0.0117$).

A number of genomic aberrations were revealed by clones that would predict a lesion as either of the two entities. The best predictor (combination of clones) showed an accuracy of 85% (Fisher's exact test, $P<0.013$). There were 87 predictor clones, the majority of which were located on chromosomes 7 and 10, plus a minority on chromosome 8 (see Supplementary Table S4 online). We also made a supervised comparison between KAs with high-grade atypia (+++) and SCCs, identifying three regions that can distinguish the two types of neoplasms, namely 10p15.3–10q21.3, 10q26.13–10q26.3, and 13q12.2–13q14.12.

Within the present study, the keratoacanthomas were classified according to their developmental stages in two ways. First, young (proliferating) and old (regressing) lesions were differentiated based on their age in weeks as estimated by the patients. Second, the degree of fibrosis and inflammation, which are associated with older lesions, was used to characterize the developmental stage. We also recorded the degree of infiltrating growth and the degree of atypia (both features shared by SCCs), the size of the lesions, and their HPV status. All these parameters were analyzed for association with the observed genetic aberrations of the KAs by *t*-test statistic analysis and permutation testing. No significant correlations were revealed between genetic aberrations and the degree of atypia, the degree of infiltration, or the estimated age of the lesions. Lesions with increased inflammation had a higher number of aberrations involving clones representing 10q23.1–10q23.32 and 13q12.11–13q32.1, whereas lesions with high levels of fibrosis selected for 3p24.3–3p14.3, and lesions with large tumor size selected for 3p26.3–3p26.1, 3p25.3–3p24.3 and 3p24.1–3q25.3 clones. HPV positivity was seen in 43 of the 75 analyzed samples (57%) and selected for several clones located on chromosome 4 (4q22.3–4q32.1 and 4q32.2–4q35.2). Furthermore, the KAs with high level of fibrosis show larger tumor size than those with low level of fibrosis (Fisher's exact test, $P<0.008$). There were no significant differences in genomic aberrations between lesions from transplanted (77) patients (immune-suppressed) and non-transplanted (21) patients.

DISCUSSION

It is well known that KAs, as well as SCCs, develop preferentially on sun-exposed areas of the skin, and that actinic keratosis as a result of sun damage is a precursor lesion for SCC (Elder *et al.*, 1997). The reported frequencies of malignant transformation within actinic keratosis lesions are debated, but recent data indicate a significant frequency of transformation, possibly >10% (Mittelbronn *et al.*, 1998). It is also reported that numerous chromosomal aberrations are shared by actinic keratosis and SCCs (Ashton *et al.*, 2003), consistent with the former lesion being a precursor. No such precursor lesion has been recognized for KAs, which (contrary to SCCs) show complete regression in practically all cases (Weedon, 2003). Despite these facts, and as there are reports of KAs giving rise to metastases, there is still a discussion whether KA is a variant of SCC (Schwartz, 2004; Karaa and Khachemoune, 2007; Ko, 2010; Weedon *et al.*, 2010a). In many cases it is difficult, especially in biopsies, to distinguish between KAs and SCCs because they share malignant features such as infiltration and atypia. Thus, it would be clinically helpful to define genetic differences that more objectively may separate the two lesions, as they require different treatments and follow-up.

In the present study, based on array CGH, all KAs were found to have detectable copy number aberrations, including the KAs (about 2/3 of the cases) that in a previous study did not show aberrations by chromosome CGH (Clausen *et al.*, 2006). This reflects the considerably higher resolution of the array method.

Recurrent aberrations, particularly on chromosomes 17, 19, 20, and X, were observed in about 1/3 of the KAs. The recurrently aberrant regions might harbor genes of particular importance for the biological features of KAs. Compared with our previous results on CGH of the same samples of KAs, the distribution of aberrations along the genome is in general similar, although the aberrations that recur most frequently were different. This is not surprising, as three times as many lesions were detected by array CGH, and, furthermore, seemingly simple gains and losses revealed by chromosome CGH have proven to consist of complex discontinuous sets of aberrations only deciphered by the high-resolution technologies (Kallioniemi, 2008).

Cytological atypia is seen in many KAs—a feature shared with SCCs. We therefore scored the degree of atypia to check whether the subgroup with atypia had certain characteristics different from lesions with less or without atypia. We did not find any differences in genetic aberrations between the groups. Furthermore, by supervised comparison between KAs with high-grade atypia (+++) and SCC, three distinguishing genomic regions were identified, namely 10p15.3–10q21.3,

10q26.13–10q26.3, and 13q12.2–13q14.12. This strongly suggests that KAs with or without atypia belong to the same subgroup, and that KAs with high-grade atypia should not be classified as SCCs based on this feature alone.

Infiltration is another feature shared between KA and SCC. There were no significant differences in genomic aberrations between KAs with little or without infiltration and those with high degree of infiltration, suggesting that this feature is consistent with the diagnosis of KA.

In all, 57% of KAs that were analyzed with respect to cutaneous and genital HPV types in the present study harbored cutaneous HPV types (Forslund *et al.*, 2003). In the present study, an association between genetic aberrations and HPV positivity was found, with the majority of selected BAC clones located on chromosome 4. Such an association is consistent with a role of HPV during KA development. However, no predominant HPV type was found in the previous study (Forslund *et al.*, 2003), which might make it less likely that HPV is causally involved in KA development.

There are three clinical stages in the typical natural history of KAs: a proliferative, maturing, and resolving stage. We have tried to characterize the stage of lesions based on the age in weeks as estimated by the patient, as well as by histopathological criteria (i.e., the degree of fibrosis and/or inflammation). No associations were found between the age in weeks and genetic aberrations or HPV status, respectively. However, the degree of fibrosis was associated with aberrations on chromosome 3 (3p24.3–3p14.3), and the degree of inflammation was associated with aberrations on chromosomes 10 (10q23.1–10q23.32) and 13 (13q12.11–13q32.1). As fibrosis and inflammation are correlated with the developmental stage, and thus the age of KAs, this result suggests that the progression of KAs may be associated with chromosomal instability and genetic changes throughout the different developmental stages. It may also indicate that more objective, histopathological criteria are better indicators of developmental stages than age estimations in weeks. However, the discrepant results may also mean that there are substantial variations in the natural length of the developmental cycle of KAs. The fact that the size of lesions was correlated with aberrations on chromosome 3 (some of which being the same as those selected for by fibrosis) is consistent with the finding that size and fibrosis are significantly associated, and that both are correlated with the late developmental stage of KAs.

Only about 16% of the analyzed SCCs failed to show genetic aberrations by chromosome CGH (Clausen *et al.*, 2006), and array CGH analysis reduced this fraction to zero. Recurring aberrations were found in >40% of SCCs on chromosomes 7, 8, 10, 13, 17, and X, with losses on certain regions of 17p and 17q recurring in >50% of samples. We have found one report in the literature on array CGH of cutaneous SCCs, showing results from eight analyzed cases, two of which were classified as crateriform/keratoacanthoma-like SCCs (Salgado *et al.*, 2008). The number of cases in that study is too small to provide reliable information on recurrent aberrations, but aberrations were found on chromosomes 7, 8, 13, and X, which was consistent with our results. Using chromosome CGH, recurrent aberrations in cutaneous SCCs were reported on chromosomes 7, 8, 17, and X by Ashton *et al.*, 2003, and aberrations on chromosome 8, 17, and X also were reported by CGH of SCCs in a review (Baudis, 2007). It is therefore reasonable to assume that aberrant genes within these recurrent regions may be determinants for the development and progression of SCCs. Recurrent aberrations on chromosomes 7, 8, 10, and 13 were not recorded in KAs, implying that genes within these regions may be responsible for the progressive growth and inability of SCCs to regress. Many aberrant genes responsible for promotion of growth and infiltration may be common for KAs and SCCs, whereas genetic aberrations promoting or making apoptosis possible have to be sought among genomic areas recurrently occurring in KAs. Candidates for such genes may be found within amplifications on chromosome X (e.g., apoptosis-inducing factor 1 and tumor necrosis factor receptor superfamily) and within amplifications on chromosome 20 (e.g., Bcl-X, death associated transcription factor 1, and retinoblastoma protein 1). The anti-apoptotic Bcl-xL protein was found to be present in 84% of SCCs compared with only 15% in KAs ($P<0.001$) (Vasiljevic *et al.*, 2009), suggesting a possible role for the anti-apoptotic Bcl-xL protein for progression and lack of regression in SCCs, and for a possible role of apoptosis in the regression of KA.

The difference in aberration patterns between KA and SCC is significant (Fisher's exact $P<0.001$) and is illustrated by the unsupervised cluster analysis in Figure 2. This is consistent with recurrent aberrations within the two types of lesions also being different, supporting the previous assumption that these lesions are separate entities (Clausen *et al.*, 2006). Our supervised cluster analysis could predict the correct classification of a lesion to an accuracy of 85% ($P<0.013$). The BAC clones (total 87 clones) of the best predictor were located on chromosomes 7, 8, and 10. This includes chromosomal regions in which many recurrent aberrations in SCCs are located, indicating that these genes, at least partly, may abrogate pathways for induction of apoptosis, or activate pathways for continuous cell proliferation and infiltration. Examples of candidate genes affected within these regions are CDC2 (cell division control protein), which appears to be deleted on chromosome 10, and GTF 21 (general transcription factor II-I), STYXL1 (Map kinase phosphatase–like protein MKSTYX), and TP53AP1 (TP53 activated protein), which all seem to be amplified on chromosome 7.

The *TP53* gene itself is of special interest in cutaneous epithelial carcinogenesis. Loss of the wild-type allele seems to be one of the earliest events in the evolutionary history of SCCs and demarcate a key transition in the progression toward cancer by unleashing broad genomic instability (Durinck *et al.*, 2011). In SCCs, loss of wild-type TP53 frequently occurs by mutation of one allele followed by copy neutral loss of heterozygosity. This form of uniparental disomy of the mutated TP53 region may represent a need to maintain some genes in diploid copy number until other genetic changes have occurred. Precursor lesions suffering a second hit in TP53 may have limited time for genetic adaptation before accumulating a catastrophic mutation load.

This window of heightened fragility could be decisive for the different fates of KAs and SCCs. The presently observed differences between average aberration values in KAs and SCCs with or without copy number loss of the TP53 region might be interpreted on this background. We can only speculate that SCCs with TP53 loss have passed the window and adapted to the genomic instability, whereas KAs with TP53 loss have not. These KAs will then regress because of new deleterious mutations to which they are not adapted. However, our array CGH analysis cannot detect copy neutral loss of heterozygosity and, thus, cannot further elucidate the hypothesis that TP53 mutations can have different effects depending on the timing relative to other genetic changes (Durinck *et al.*, 2011).

If we assume that KA and SCC are biologically separate lesions with different pathogenetic pathways, how do we explain the presence of overlapping genetic imbalances detected by array CGH? First of all, many, if not most, of the aberrations detected are probably without influence on tumor progression and apoptosis/cell death. Second, it is well known that sun exposure through UV irradiation is important for the development of KAs and SCCs. Thus, many of the overlapping aberrations may be due to DNA damage caused by the common influence of UV. Considering the suggestion that SCCs may develop with low frequency in KAs of elderly persons (Weedon *et al.*, 2010b), some overlap of genetic aberrations may also be expected in some cases. The observation that genetic aberrations are more numerous in SCC than in KA may cause abrogation of many pathways in SCC that are intact in KA, allowing apoptosis and spontaneous regression of the latter.

Chromosome CGH and array CGH are screening methods with the advantage that they allow for simultaneous detection and mapping of amplified and deleted chromosomal regions of the entire genome of tumors in one single hybridization (Kallioniemi *et al.*, 1992; Pinkel *et al.*, 1998). Limitations are that balanced aberrations, such as translocations and inversions, cannot be detected, and that the hybridization target (chromosomes/arrays of BAC clones representing the relative genomic sequences) limits the resolution of the methods. However, array CGH data are significantly contributing by suggesting genomic regions of interest for further search for genes of importance for tumor classification before massively parallel sequencing of captured regions or whole tumor genomes is becoming practically feasible.

MATERIALS AND METHODS

Patients

A total of 132 KAs and 29 SCCs from the skin were collected in the period from1995 to 2004. Among them, 126 KAs and 18 SCCs were from patients consulting the Departments of Dermatology, Surgery and Plastic surgery at Rikshospitalet, University of Oslo. DNA extracted from lesions of 17 patients, 6 with KAs and 11 with SCCs, were kindly provided from Center for Cutaneous Research, University of London UK. DNA from these lesions, and from those collected at Rikshospitalet, University of Oslo, was analyzed by CGH as previously reported (Clausen *et al.*, 2006). There was sufficient remaining DNA from 98 KAs and 22 SCCs from this material to allow further analysis by array CGH. The project was approved by the ''Regional committee for medical research ethics Sothern-Norway (REK Sør)''. The project number is S-06158. All patients gave written, informed consent in adherence with the Declaration of Helsinki Principles. The corresponding information of the histopathological evaluation and HPV analysis for the 120 samples is provided in ''Supplementary Materials and Methods online.'' The summary of the clinicopathological description for the 98 KAs is presented in Supplementary Table S1 online.

Array CGH and related data analysis

The array CGH analysis was performed as previously described (Li *et al.*, 2008, 2009). The detailed information of experimental work and data analysis is provided in ''Supplementary Materials and Methods online.''

CONFLICT OF INTEREST

The authors state no conflict of interest.

ACKNOWLEDGMENTS

We are thankful for the support from the project ''Molecular Tools for Optimal Personalized Treatment of Breast Cancer'' under the auspices of the Sino-Danish Breast Cancer Research Centre, financed by the Danish ''Grundforskningsfonden'' and ''The Natural Science Foundation of China''. Support was also received from the project ''Molecular Tools for Optimal Personalized Treatment of Colorectal Cancer'' (FØSU), Danish Centre for Translational Breast Cancer Research (DCTB), ''A Race Against Breast Cancer'' (ARABC), the OAK Foundation, and ''Den Bøhmske Fond''. We also thank Helse Sør-Øst and Astrid and Birger Torsteds legacy for financial support.

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/jid

REFERENCES

Ashton KJ, Weinstein SR, Maguire DJ *et al.* (2003) Chromosomal aberrations in squamous cell carcinoma and solar keratoses revealed by comparative genomic hybridization. *Arch Dermatol* 139:876–82

Baudis M (2007) Genomic imbalances in 5918 malignant epithelial tumors: an explorative meta-analysis of chromosomal CGH data. *BMC Cancer* 7:226

Clausen OP, Aass HC, Beigi M *et al.* (2006) Are keratoacanthomas variants of squamous cell carcinomas? A comparison of chromosomal aberrations by comparative genomic hybridization. *J Invest Dermatol* 126:2308–15

Clausen OP, Beigi M, Bolund L *et al.* (2002) Keratoacanthomas frequently show chromosomal aberrations as assessed by comparative genomic hybridization. *J Invest Dermatol* 119:1367–72

Cribier B, Asch P, Grosshans E (1999) Differentiating squamous cell carcinoma from keratoacanthoma using histopathological criteria. Is it possible? A study of 296 cases. *Dermatology* 199:208–12

Durinck S, Wang N, Liao W *et al.* (2011) Temporal dissection of tumorigenesis in primary cancers. *Cancer Discov* 1:137–43

Elder DER, Jaworsky C, Johnson Jr B (eds) (1997) *Lever's Histopathology of the Skin*, 8th edn. Lippincott-Raven Publishers: Philidelphia

Ferguson-Smith MA, Wallace DC, James ZH *et al.* (1971) Multiple self-healing squamous epithelioma. *Birth Defects Orig Artic Ser* 7:157–63

Forslund O, DeAngelis PM, Beigi M *et al.* (2003) Identification of human papillomavirus in keratoacanthomas. *J Cutan Pathol* 30:423–9

Grzybowski M (1950) A case of peculiar generalized epithelial tumors of the skin. *Br J Dermatol* 62:310–3

Hodak E, Jones RE, Ackerman AB (1993) Solitary keratoacanthoma is a squamous-cell carcinoma: three examples with metastases. *Am J Dermatopathol* 15:332–42

Jensen P, Clausen OP, Bryne M (1999) Differences in sialyl-Tn antigen expression between keratoacanthomas and cutaneous squamous cell carcinomas. *J Cutan Pathol* 26:183–9

Kallioniemi A (2008) CGH microarrays and cancer. *Curr Opin Biotechnol* 19:36–40

Kallioniemi A, Kallioniemi OP, Sudar D *et al.* (1992) Comparative genomic hybridization for molecular cytogenetic analysis of solid tumors. *Science* 258:818–21

Karaa A, Khachemoune A (2007) Keratoacanthoma: a tumor in search of a classification. *Int J Dermatol* 46:671–8

Kerschmann RL, McCalmont TH, LeBoit PE (1994) p53 oncoprotein expression and proliferation index in keratoacanthoma and squamous cell carcinoma. *Arch Dermatol* 130:181–6

Ko CJ (2010) Keratoacanthoma: facts and controversies. *Clin Dermatol* 28:254–61

Li J, Gromov P, Gromova I *et al.* (2008) Omics-based profiling of carcinoma of the breast and matched regional lymph node metastasis. *Proteomics* 8:5038–52

Li J, Wang K, Li S *et al.* (2009) DNA copy number aberrations in breast cancer by array comparative genomic hybridization. *Genomics Proteomics Bioinform* 7:13–24

Mandrell JC, Santa Cruz D (2009) Keratoacanthoma: hyperplasia, benign neoplasm, or a type of squamous cell carcinoma? *Semin Diagn Pathol* 26:150–63

Mittelbronn MA, Mullins DL, Ramos-Caro FA *et al.* (1998) Frequency of pre-existing actinic keratosis in cutaneous squamous cell carcinoma. *Int J Dermatol* 37:677–81

Muir EG, Bell AJ, Barlow KA (1967) Multiple primary carcinomata of the colon, duodenum, and larynx associated with kerato-acanthomata of the face. *Br J Surg* 54:191–5

Pinkel D, Segraves R, Sudar D *et al.* (1998) High resolution analysis of DNA copy number variation using comparative genomic hybridization to microarrays. *Nat Genet* 20:207–11

Putti TC, Teh M, Lee YS (2004) Biological behavior of keratoacanthoma and squamous cell carcinoma: telomerase activity and COX-2 as potential markers. *Mod Pathol* 17:468–75

Quinn AG, Sikkink S, Rees JL (1994) Basal cell carcinomas and squamous cell carcinomas of human skin show distinct patterns of chromosome loss. *Cancer Res* 54:4756–9

Salgado R, Toll A, Espinet B *et al.* (2008) Analysis of cytogenetic abnormalities in squamous cell carcinoma by array comparative genomic hybridization. *Actas Dermosifiliogr* 99:199–206

Schwartz RA (1994) Keratoacanthoma. *J Am Acad Dermatol* 30:1–19

Schwartz RA (2004) Keratoacanthoma: a clinico-pathologic enigma. *Dermatol Surg* 30:326–33

Snijders AM, Nowak N, Segraves R *et al.* (2001) Assembly of microarrays for genome-wide measurement of DNA copy number. *Nat Genet* 29:263–4

Sullivan JJ, Colditz GA (1979) Keratoacanthoma in a sub-tropical climate. *Australas J Dermatol* 20:34–40

Vasiljevic N, Andersson K, Bjelkenkrantz K *et al.* (2009) The Bcl-xL inhibitor of apoptosis is preferentially expressed in cutaneous squamous cell carcinoma compared with that in keratoacanthoma. *Int J Cancer* 124:2361–6

Weedon D (2003) Keratoacanthoma: a personal perspective. *Curr Diagn Pathol* 9:259–65

Weedon D, Malo J, Brooks D *et al.* (2010a) Keratoacanthoma: is it really a variant of squamous cell carcinoma? *ANZ J Surg* 80:129–30

Weedon DD, Malo J, Brooks D *et al.* (2010b) Squamous cell carcinoma arising in keratoacanthoma: a neglected phenomenon in the elderly. *Am J Dermatopathol* 32:423–6